CLAIMS

1. A crystal comprising a mannosidase II ligand-binding domain.

2. A crystal according to claim 1, which is a crystal of a mannosidase II.

3. A crystal according to claim 2 characterized by an N-terminal α/β domain, a C-terminal portion comprising a three-helical bundle, and an all-β C-terminal domain, connected by 5 internal disulfide bonds and stabilized by a zinc binding site.

4. A crystal according to claim 3 wherein the N-terminal α/β domain is characterized by the following:
   (a) comprising an inner core of three β-sheets (A, B and C, Figure 8B) consisting of 11, mostly parallel β-strands, surrounded by 16 α-helices;
   (b) comprising a GlcNAc residue at a consensus N-glycosylation site (Asn-194), located at the N-terminus of helix 7; and
   (c) stabilized by three disulfide bonds: between Cys-31 and Cys-1032 connecting the N and C-terminal extremes of dGMII; Cys-275 and Cys-282 linking helices 10 and 11; Cys-283 and Cys-297 linking helix 11 with a loop between helix 13 and the core of parallel β-sheets.

5. A crystal according to claim 3 wherein the C-terminal portion is characterized by the following:
   (a) a three-helix bundle comprises helices 18, 20 and 21 connected to the N-terminal α/β-domain via a zinc binding site;
   (b) a zinc ion coordinated in a T_5-square-based pyramidal geometry involving residues: Asp-90, His-92, Asp-204 and His-471;
   (c) two immunoglobulin-like domains: a small β-sandwich consisting of 12 anti-parallel strands from β-sheets D and E, and a large 21-strand structure involving β-sheets F and G; and

(d) a barrel formed by the three-helix bundle, helix-23, and the two β-sandwich structures provides a narrow pore in the center of the C-terminal domain.

6. A crystal according to claim 1 or 2, comprising a complex between a mannosidase II ligand-binding domain and at least one ligand.

7. A crystal according to claim 3, wherein the ligand is swainsonine or a derivative thereof.

8. A crystal as claimed in claim 2 which is characterized by the following:
(a) a small cavity lined by aromatic residues Trp-95, Phe-206, Tyr-269 and Tyr-727;
(b) a zinc ion binding site within the cavity characterized by a T_5-square-based pyramidal geometry and 'elec-His-Zn motifs'.

9. A crystal as claimed in claim 1 wherein the ligand binding domain comprises one or more of amino acid residues Trp-95, Phe-206 and Tyr-727 which form a binding cavity for a mannosidase II inhibitor.

10. A crystal as claimed in claim 1 wherein the ligand binding domain is capable of binding a zinc ion characterized by a T_5-square-based pyramidal geometry involving amino acid residues: Asp-90, His-92, Asp-204 and His-471

11. A crystal as claimed in claim 1 wherein the ligand binding domain comprises one or more of amino acid residues: His 471, His 90, and Asp 92, and Asp 204; or a homologue thereof

12. A crystal as claimed in claim 1 wherein the ligand binding domain comprises one or more of amino acid residues: Trp-95, Phe-206, Tyr-269, and Tyr-727.

13. A crystal as claimed in claim 1 wherein the ligand binding domain comprises one or more of amino acid residues: Asp-92, Asp-204, His-90, His-471.

14. A crystal according to claim 1 wherein said ligand-binding domain comprises one or more of the following residues: His 471, Asp 204, Asp 341, His 90, Asp 92, Asp 472, Phe 206, Tyr 727 and Tyr 95.

15. A crystal according to claim 1 which comprises one or more of the residues shown in Table 3 or 4.

16. A crystal according to claim 1 wherein said ligand-binding domain comprises one or more of the following groups:

(a) GVWKQG (residues 60-65)

(b) VFVVPHSHND (residues 83-92)

(c) WAIDPFGH (residues 201-208)

(d) HMMPFYSYDIPHTCGPDPK$^{V}/_{I}$CCQFDFKR (residues 262-289)

(e) LL$^{I}/_{A}$PLGDDFR (residues 334-343)

17. A crystal according to any preceding claim, wherein the crystal has $P2_1$ symmetry.

18. A crystal according to any preceding claim, wherein said crystal comprises a unit cell having the following dimensions: a=69 (±5) Å, b=110 (±5) Å, c=139 (±5) Å.

19. A crystal according to any preceding claim having the structural coordinates as shown in Table 1, Table 2, or Table 8.

20. A crystal according to claim 2 comprising one or more of a cofactor, a mannosidase II inhibitor, or a substrate.

21. A crystal of a mannosidase II according to claim 2 defined by the interactions of Table 4.

22. A crystal comprising swainsonine or a derivative thereof having the structural coordinates as shown in Table 2 or Table 8.

23. A computer readable medium having stored thereon: the structure of a crystal according to any of claims 1 to 21.

24. Machine readable media encoded with data representing the structural coordinates of a crystal or ligand binding domain according to any of the preceding claims.

25. A method of screening for a ligand capable of binding a mannosidase II ligand binding domain, comprising the use of a crystal according to any of claims 1 to 21.

26. A method of screening for a ligand according to claim 25, which comprises the step of contacting the ligand binding domain with a test compound, and determining if said test compound binds to said ligand binding domain.

27. A ligand identified by a method according to claim 25 or 26.

28. A ligand according to claim 27, which is capable of interacting with one or more of the residues of a mannosidase II shown in Table 3 or 4.

29. A modulator of the activity of a mannosidase II derived from a crystal as claimed in any of the preceding claims.

30. A method for identifying a potential modulator of a mannosidase II, or ligand binding domain thereof, comprising the step of using the structural coordinates of Table 1, 2, or 8 that define a mannosidase II or ligand binding domain thereof, to computationally

evaluate a test compound for its ability to associate with the mannosidase II or ligand binding domain, wherein a test compound that associates is a potential modulator of a mannosidase II.

31. A method for identifying a modulator of a mannosidase II by determining binding interactions between a test compound and binding site of a ligand binding domain of a mannosidase II as defined in Table 4 comprising:

(a) generating the binding site on a computer screen;

(b) generating a test compound with its spatial structure on the computer screen; and

(c) testing to determine whether the test compound binds to a selected number of atomic contacts in a binding site.

32. A method for identifying a potential modulator of a mannosidase II function comprising the steps:

(a) docking a computer representation of a test compound from a computer data base with a computer representation of a crystal of a mannosidase II as claimed in the preceding claims, to obtain complexes;

(b) determining conformations of complexes with a favourable geometric fit and favourable complementary interactions; and

(c) identifying a conformation of a compound that best fits the selected site as a potential modulators of the mannosidase II.

33. A method for identifying a potential modulator of a mannosidase II function comprising the steps:

(a) modifying a computer representation of a test compound complexed with a crystal of a ligand binding domain of a mannosidase II as described in any of the preceding claims, by deleting or adding a chemical group or groups;

(b) determining a conformation of the complex with a favourable geometric fit and favourable complementary interactions; and

(c) identifying a compound that best fits the binding site as a potential modulator of a mannosidase II.

34. A method for identifying a potential modulator of a mannosidase II function co comprising the steps:

(a) selecting a computer representation of a test compound complexed with a crystal of a ligand binding domain of a mannosidase II as defined in the preceding claims; and

(b) searching for molecules in a data base that are similar to the test compound using a searching computer program, or replacing portions of the test compound with similar chemical structures from a data base using a compound building computer program.

35. A modulator of a mannosidase II identified by a method according to any of the preceding claims.

36. A modulator of a mannosidase II based on the three-dimensional structure of an inhibitor's spatial association with a crystal as claimed in any of the preceding claims.

37. A method for designing potential inhibitors of a mannosidase II comprising the step of using the structural coordinates of a mannosidase II inhibitor defined in relation to its spatial association with a crystal of a mannosidase II or a ligand binding domain thereof according to any of the preceding claims, to generate a compound that is capable of associating with the mannosidase II or ligand binding domain thereof.

38. The use of a ligand according to claim 27 or 28, in the manufacture of a medicament to treat and/or prevent a disease in a mammalian patient.

39. A pharmaceutical composition comprising a ligand according to any of claims 27 or 28 and optionally a pharmaceutically acceptable carrier, diluent, excipient or adjuvant or any combination thereof.

40. A pharmaceutical composition comprising a modulator according to any of the preceding claims either alone or with other active substances.

41. A method of treating a disease associated with a mannosidase II in a cellular organism, comprising:

(a) administering a pharmaceutical composition according to claim 39 or 40; and

(b) activating or inhibiting a mannosidase II to treat the disease.

42. A method of treating and/or preventing a disease comprising administering a ligand according to claim 27 or 28 and/or a pharmaceutical composition according to claim 39 or 40 to a mammalian patient.

43. A method of determining the secondary and/or tertiary structures of a polypeptide with unknown structure comprising the step of using a crystal according to any of claims 1 to 21.

44. Plasmid pCopBlast.

45. A host cell comprising a plasmid as claimed in claim 44.

46. A method for preparing a mannosidase II using a plasmid as claimed in claim 44.

47. A method for preparing a mannosidase II is provided comprising:

(a) transferring a plasmid as claimed in claim 44, into a host cell;

(b) selecting transformed host cells from untransformed host cells;

(c) culturing a selected transformed host cell under conditions which allow expression of the mannosidase II and

(d) isolating the mannosidase II.